EFFECTS OF DIETARY *Diospyros mespiliformis* LEAF AND STEM-BARK EXTRACTS ON GROWTH PERFORMANCE AND INTESTINAL MORPHOMETRICS IN *Clarias gariepinus*

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ABSTRACT
The global criticism due to the deleterious effects of applying synthetic drugs in the production of food fish necessitates eco-friendly alternatives, like African ebony (AE). The AE have been reported to possess antimicrobial and antioxidant properties, but there is limited information on its application in aquaculture. Hence, this study investigated the phytochemical components and the effects of dietary supplementation with *AE* extracts on the growth performance and intestinal morphometrics of *Clarias gariepinus*. A preliminary phytochemical assessment was done, after which 240 fish fingerlings were randomly distributed into 12 tanks and fed with isonitrogenous and isocaloric basal diets containing 0.0, 0.5 leaf, 0.5 stem-bark, or 0.5 mL combined leaf and stem-bark ethanol extracts/100 g diet to apparent satiation for eight weeks. After the feeding trial, intestinal morphometrics were measured using standard procedures. Data obtained were analyzed using one-way analysis of variance at $P < 0.05$. Highest flavonoids and lower tannins were observed in AE ethanol extracts. Fish fed stem-bark extract exhibited significantly higher final weight, weight gain, specific growth rate, protein efficiency ratio, and lower feed conversion ratio, compared to other treatments. The fish fed diets supplemented with AE stem-bark also showed higher villi heights and width at the proximal and distal sections of the intestine, compared to other treatments. Therefore, 0.5 mL AE stem-bark/100 g diet is recommended for the production of *C. gariepinus*.

Keywords: *Diospyros mespiliformis, African catfish, growth performance, intestinal morphometrics*

INTRODUCTION

Aquaculture is the fastest-growing animal-food producing sector worldwide and plays an essential role in food security and livelihoods, particularly across sub-Saharan Africa, where the African catfish (*Clarias gariepinus*) is a major cultured species because of its fast growth, wide environmental tolerance, and market value (FAO, 2024; Adeniyi *et al.*, 2025). However, intensified culture systems have elevated disease incidence, increased dependence on synthetic drugs, leading to serious global concerns about its residual effects, antimicrobial resistance, food and environmental safety (Adeniyi *et al.,* 2023). These challenges have driven research into sustainable health-promoting alternatives for aquafeeds, including probiotics, prebiotics, and increasingly, plant-derived phytobiotics (herbal extracts and essential oils). Phytobiotics have been reported to be eco-friendly growth-promoters and immunostimulants in finfish and shellfish farming (Adeniyi *et al*., 2022; Kolygas *et al.,* 2025).

Phytobiotics are rich in secondary metabolites, including flavonoids, tannins, saponins, alkaloids, terpenes and phenolics, that exert antioxidant, antimicrobial, and immunomodulatory activities and can improve feed utilization, gut physiology, and stress resilience in cultured fish (Radwan *et al.,* 2022; Sandeep *et al.,* 2025). Experimental studies across a range of species demonstrated that dietary inclusion of phytobiotics in the form of powder or concentrated extracts often enhance growth performance, feed efficiency, and intestinal histomorphometry, which are central to sustainable production and reduced antibiotic reliance (Adegbesan *et al.,* 2020; Radwan *et al.,* 2022; Adeniyi *et al.*, 2023; Kolygas *et al.,* 2025).

Diospyros mespiliformis (Jackalberry, Ebenaceae), also known as African ebony (AE), is a widespread African tree with documented traditional medicinal uses and multiple pharmacological properties, including antioxidant, antimicrobial, and anti-inflammatory activities. The *in vitro* study reported by Mustapha *et al.* (2022) confirmed the antioxidant and antimicrobial potential of *D. mespiliformis* extracts and highlighted low acute toxicity in standard assays, supporting its candidacy as a safe phytobiotic source. The leaves and bark of the plant is a rich source of both primary and secondary metabolites that could be responsible for it ethnopharmacological uses for the treatments of cough, diarrhea, dysentery, fever, stomach aches, wounds, among others, in different regions of Africa (Ramadwa and Meddows-Taylor, 2023). Based on the identified potentials of AE, the herbal resource could improve the growth performance in fish. Despite these promising phytochemical and bioactivities of AE, there is limited information on its assessment in farmed fishes. Accordingly, this study evaluated the effects of dietary supplementation with AE leaf and stem-bark extracts on growth performance and intestinal morphometry of *C. gariepinus*.

MATERIALS AND METHODS

Plant source and extraction

Fresh leaves and stem-bark of African ebony (AE) were obtained from the National Institute for Freshwater Fisheries Research (NIFFR) Mini Forest in New Bussa, Niger State. The leaves and stem-bark were cleaned with sterile water, air-dried at room temperature for 14 days, and thereafter ground to powder form using a kitchen blender. Each of the fine powdered plant leaf and stem-bark was weighed using an electronic weighing balance and mixed with ordinary distilled water at ambient temperature (25 °C), warm distilled water at 50 °C and ethanol at the ratio of 1:5 (weight of plant/volume of solvent). The extraction was done for 72 h, during which the mixed samples were allowed to shake for 24 h on an electrical orbital shaker (DLAB, SK0330 Pro, United Kingdom) at an appropriate speed of 4000 rpm and thereafter intermittently agitated. The warm water extraction was placed in a water bath at 50 °C. After the extraction time, each sample was filtered using a double layer of muslin cloth and then through Whatman filter paper. The extracts were concentrated using a rotator evaporator (Stone Staffordshire, UK), and the concentrated AE leaf and stem-bark extracts, were stored in freezer before use (Adeniyi *et al*. 2021).

Qualitative and quantitative phytochemical screening

The qualitative screening of AE leaf and stem-bark extract was carried out for the presence of major secondary metabolites (tannins, saponins, flavonoids, terpenoids, steroids, alkaloids, reducing sugars) was assessed using standard colorimetric and precipitation tests, while the quantitative screening were also done for saponins, flavonoids, tannins and alkaloids by precipitation, gravimetric or spectrophotometry procedures described by Harborne (1998).

Experimental fish and culture

Clarias gariepinus fingerlings were obtained from a reputable farm, in Ilorin, Nigeria and acclimated for 7 days, during which they were fed with commercial feed (1.8 mm, Skretting) before the commencement of feeding trial. Thereafter, 240 pieces of fish were randomly distributed among the 12 rectangular plastic tanks (80 cm × 60 cm × 45 cm) at a rate of 20 fish/tank. The AE extracts were added to the commercial diet at 0.0 (Control), 0.5 leaf, 0.5 stem-bark, and combined (50:50) 0.5 leaf + stem-bark mL/100 g basal diet, mixed, shade-dried, packed in polythene bags, labelled, and stored. The fish were given the specified diet twice daily until they appeared satisfied for eight weeks, while the diets were reproduced biweekly during feeding trial. The weights of the fish and the feed provided to them were recorded throughout the experiment. To maintain good water quality, the water in each experimental tank was completely replenished once every 48 hours. The pH, dissolved oxygen and temperature were measured using dissolved oxygen meters (AMT07; C. V. Java Multi Mandiri, Indonesia) and were found to be within the range of 7.0-7.2, 4.7-5.3mg/L of water, and 26.6-27.7ºC, respectively, which are within the recommended ranges for aquaculture (Boyd and Tucker, 2012).

Evaluation of growth performance and nutrient utilization

After the eight weeks feeding trial, fish from each tank were batch-weighed and the following growth performance, feed utilization and survival parameters were calculated:

Weight gain (WG, g) = Final weight (FW) – Initial weight (IW)

Specific growth rate (SGR, %/day) = 100 (Ln FW- Ln IW) / Duration of experiment (days)

Feed intake (g/fish) = Sum of the feed consumed by fish in a replicate / Number of fish

Feed conversion ratio (FCR) = Feed intake / WG

Protein efficiency ratio (PER) = WG / Protein intake\ *Note: Protein intake (g) = Crude protein in diet × Feed intake*

Fish survival (%) = 100 x (Number of survived fish / Initial number of fish stocked)

Nitrogen metabolism (g) = Experimental period (days) × (0.549) × (IW + FW) / 2

Where, 0.549 is a constant (Dabrowski, 1977)

Fish productivity index = (Weight gain × Fish survival) / (Feed conversion ratio × 10) (Alatore-Jacome *et al.,* 2012).

Intestinal morphometry

Fish were starved for 24 hours after the feeding experiment to make sure their guts were empty of food and their anterior intestines were removed and the proximal, mid, and distal regions were identified and cut (Anguiano *et al*. 2013). The approach for a routine histological process was used to prepare the intestinal sections (Takashima and Hibiya 1995). Briefly, the intestines regions were fixed in 10% buffered formalin, dehydrated with series alcohol concentrations, cleared with xylene, embedded in molten paraffin wax, sectioned to a thickness of 5 um, stained with hematoxylin and eosin on slides, and viewed under a light microscope to measure the villus height, width and cryptal width, using a binocular microscope (Olympus Cx21, Tokyo, Japan), a micrometer rule, and a digital camera. The villi area of absorption was calculated by multiplying villus height and width (Adeniyi *et al.,* 2023).

Data analysis

The data obtained were analyzed using one-way analysis of variance, while Duncan's multiple range test was employed to distinguish group means following a significant ANOVA result ($P < 0.05$). The analysis was carried out using IBM SPSS version 25.

RESULTS

Phytochemical components of African ebony leaves and stem-bark extract

The results of the qualitative phytochemical screenings showed that tannins, saponins, and alkaloids were consistently present across all the AE extracts (Table 1). The AE leaf extracts showed higher levels of tannins, saponins and alkaloids. Reducing sugars were highest in the aqueous warm extracts, while triterpenoids were absent in all samples. Qualitatively, the AE stem-bark extracts showed elevated levels of flavonoids, quinine,

saponins, and reducing sugars, particularly in the aqueous warm and ethanol extracts. The results on the quantitative phytochemical screening of AE leaf and stem-bark extracts are presented in Table 2. The concentration of saponins, tannins and alkaloids were significantly reduced in the stem-bark extracts, compared with the leaf extracts. The highest ($P < 0.05$) flavonoid concentration was found in the ethanol extracts, followed by the aqueous warm extracts, while the least was found in ordinary aqueous extracts. Significantly higher tannins and alkaloids were observed in aqueous warm leaf extract, compared with other extracts. The quantities of the four phytochemicals were lower ($P < 0.05$) in the ordinary aqueous extracts of both the leaf and the stem-bark, compared to aqueous warm and ethanol extracts.

Growth performance and nutrient utilization

Among the four treatments, the fish fed the AE stem-bark extract demonstrated higher ($P < 0.05$) weight gain, specific growth rate, protein efficiency ratio (PER), and significantly lower feed conversion ratio (FCR), compared to other treatments (Table 3). Although the highest feed intake was obtained in the fish fed the control diet, the values of FCR and PER did not differ ($P < 0.05$) from values obtained for the fish 0.5 mL AE leaf. All the experimental fish in the various treatments survived (100%). The lowest ($P < 0.05$) nitrogen metabolism and fish performance index were obtained in the fish fed 0.5 mL AE leaf, compared to other treatments.

Table 1: Qualitative phytochemical components of African ebony leaves and stem-bark extract

Phytochemical components	Leaf extracts			Stem-Bark extracts		
	ALE	AWLE	ELE	ASE	AWSE	ESE
Flavanoids	++	++	++	++	++	+++
Phenolics	++	++	++	+	++	++
Quinine	+	+	+	+	+++	+++
Steriods	++	++	++	-	++	++
Triterpenoids	-	-	-	-	-	-
Cardiac Glycosides	++	++	++	+	+	++
Tannins	+++	+++	+++	+	++	++
Saponins	+++	+++	+++	+	+++	++
Alkaloids	+++	+++	+++	++	++	++
Reducing Sugar	-	+++	++	++	+++	+++
Phlobatanins	+	+	+	+	+	+
Terpenoids	+	+	+	-	+	+

ALE, Aqueous leaf extract; AWLE, Aqueous warm leaves extract; ELE, Ethanol leaves extract; ASE, Aqueous stem-bark extract, AWSE, aqueous warm stem-bark extract; ESE, Ethanol stem-bark extract.

Table 2: Quantity of some phytochemical components of African ebony leaf and stem-bark extracts

Extracts	Phytochemicals (g/100 g)			
	Saponins	Flavonoids	Tannins	Alkaloids
ALE	0.0168 ± 0.00^{c}	0.0547 ± 0.00^{e}	0.0370 ± 0.00^{c}	0.0480 ± 0.00^{b}
AWLE	0.0177 ± 0.00^{b}	0.0653 ± 0.00^{c}	0.0480 ± 0.00^{a}	0.0593 ± 0.00^{a}
ELE	0.0183 ± 0.00^{a}	0.0693 ± 0.00^{b}	0.0413 ± 0.00^{b}	0.0433 ± 0.00^{c}
ASE	0.0034 ± 0.00^{f}	0.0413 ± 0.00^{f}	0.0068 ± 0.00^{f}	0.0221 ± 0.00^{f}
AWSE	0.0080 ± 0.00^{d}	0.0580 ± 0.00^{d}	0.0216 ± 0.00^{e}	0.0251 ± 0.00^{e}
ESE	0.0059 ± 0.00^{c}	0.0705 ± 0.00^{a}	0.0269 ± 0.00^{d}	0.0291 ± 0.00^{d}

Means with different superscripts on the same column are significantly different at $P < 0.5$

ALE, Aqueous leaves extract; AWLE, Aqueous warm leaves extract; ELE, Ethanol leaf extract; ASE, Aqueous stem-bark extract; AWSE, Aqueous warm stem-bark extract; ESE, Ethanol stem-bark extract.

Table 3: Growth performance and nutrient utilization of *Clarias gariepinus* fed diets supplemented with African ebony leaf and stem-bark extracts

Parameters	Treatments and dietary levels (mL/100 g diet)			
	0.0 (Control)	0.5 Leaf	0.5 Stem-bark	0.5 Leaf + Stem-bark
IW (g)	3.10±0.03^{a}	3.05±0.06^{a}	3.05±0.03^{a}	2.95±0.05^{a}
FW (g)	15.86±0.04^{b}	13.64±0.03^{c}	16.69±0.16^{a}	15.80±0.15^{b}
WG (g)	12.76±0.05^{b}	10.59±0.07^{c}	13.64±0.16^{a}	12.85±0.18^{b}
PWG (%)	511.66±5.16^{b}	513.22±9.97^{b}	547.27±7.53^{a}	536.07±12.07^{ab}
SGR (%/day)	2.92±0.02^{b}	2.92±0.03^{b}	3.03±0.02^{a}	3.00±0.04^{ab}
FI (g)	16.25±0.14^{a}	13.80±0.12^{c}	15.40±0.22^{b}	15.65±0.20^{b}
FCR	1.27±0.01^{a}	1.30±0.01^{a}	1.13±0.01^{c}	1.22±0.01^{b}
PER	1.51±0.01^{c}	1.48±0.02^{c}	1.70±0.01^{a}	1.58±0.01^{b}
FS (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
NM	291.4±0.72^{b}	256.58±0.90^{c}	303.42±2.43^{a}	288.27±2.22^{b}
FPI	100.18±0.23^{c}	81.31±1.23^{d}	120.79±1.78^{a}	105.59±1.99^{b}

Means with different superscript on the same row are significantly different at $P < 0.05$
IW, Initial weight; FW, Final weight; WG, Weight gain; PWG, Percentage weight gain; SGR, Specific growth rate; FI, Feed intake; FCR, Feed conversion ratio; PER, Protein efficiency ratio; NM, Nitrogen metabolism; FPI, Fish performance index.

Intestinal morphometrics

The intestinal villi heights of *C. gariepinus* fed AE leaf, stem-bark or combined leaf and stem-bark extracts are presented in Figure 1. The villi heights reduced from proximal section through the distal section of the intestine. The fish fed diets supplemented with AE stem-bark showed higher ($P < 0.05$) villi heights at the proximal (2263.64±3.08) and mid (2236.06±11.99) sections of the intestines, compared with those of the fish fed with other diets. There were no significant differences of the villi heights at the distal section.

The intestinal villi widths of *C. gariepinus* fed AE leaf, stem-bark or combined leaf and stem-bark extracts are presented in Figure 2. The highest ($P < 0.05$) villi width was obtained at the intestinal proximal and distal sections of the fish fed AE stem-bark, while value obtained in the fish fed the control diet were higher than those fed the AE leaf and the leaf + stem-bark extracts. However, there were no ($P > 0.05$) significant differences in the villi width at the mid-sections of the intestine. Furthermore, the fish fed the stem-bark extract had higher villi width at the distal region, compare with the other treatments.

The highest ($P < 0.05$) intestinal villi absorption area at the proximal region was obtained in *C. gariepinus* fed AE stem-bark extract, followed by the fish fed the control diet, and then the treatment fed with the combined leaf and stem-bark extracts, while the least was obtained in the fish fed the leaf extract (Figure 3). Similar ($P > 0.05$) villi absorption areas were obtained at the mid-section of the intestine across the treatment, except in the fish fed 0.5 mL AE leaf extract / 100 g diet that exhibited lower ($P < 0.05$) absorption area. Higher ($P < 0.05$) villi absorption area was also obtained at the intestinal distal sections of the fish fed AE stem-bark, compared with other treatments.

Similar ($P > 0.05$) villi cryptal widths were obtained across the treatments at the proximal and mid-sections of the intestine, except in the fish fed 0.5 mL AE leaf extract / 100 g diet that exhibited lower ($P < 0.05$) values (Figure 4). Higher ($P < 0.05$) cryptal widths were obtained at the intestinal distal section of the fish fed AE stem-bark and the control diets, compared with the fish fed other diets.

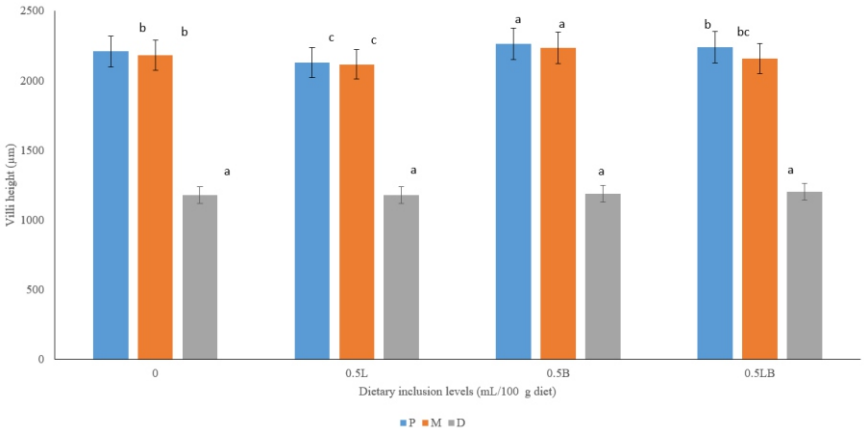

Figure 1: Villi height of *Clarias gariepinus* fed diets supplemented with African ebony leaf and stem-bark extracts for 8 weeks
Similar bars with different letters are significantly different at $P < 0.05$
L, Leaf; B, Bark; LB, Leaf + Stem-Bark; P, Proximal region; M, Mid-region; D, Distal region

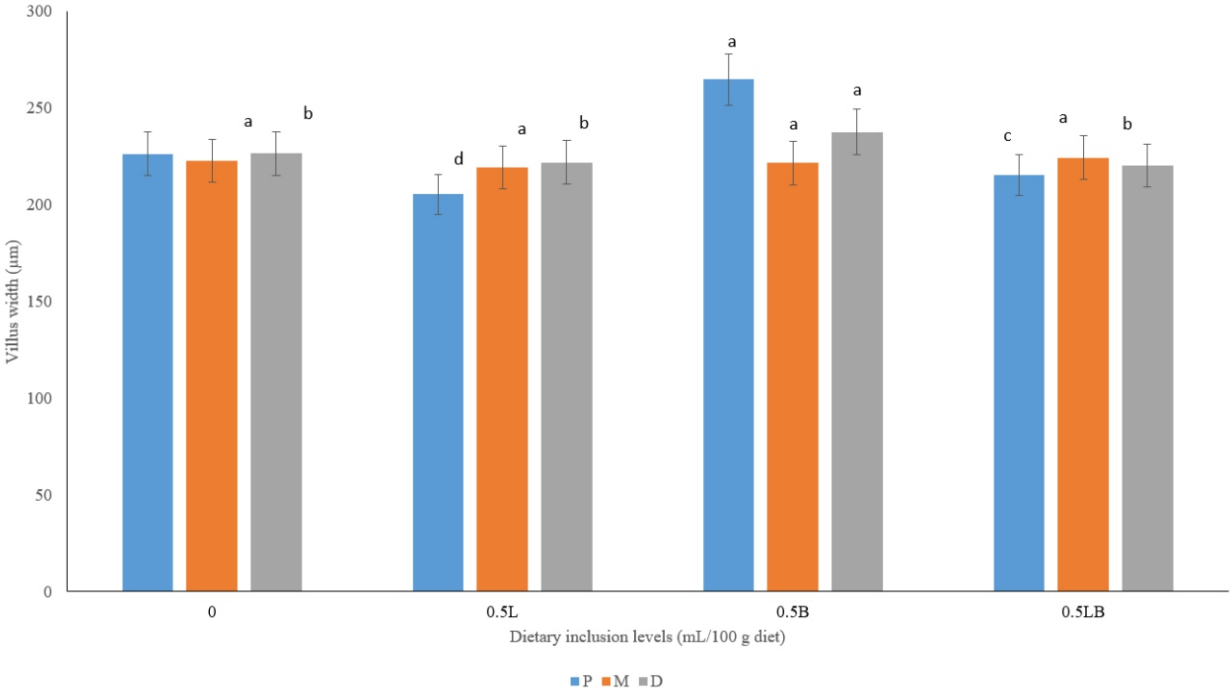

Figure 2: Villi width of *Clarias gariepinus* fed diets supplemented with African ebony leaf and stem-bark extracts for 8 weeks

Similar bars with different letters are significantly different at $P < 0.05$
L, Leaf; B, Bark; LB, Leaf + Stem-Bark; P, Proximal region; M, Mid-region; D, Distal region

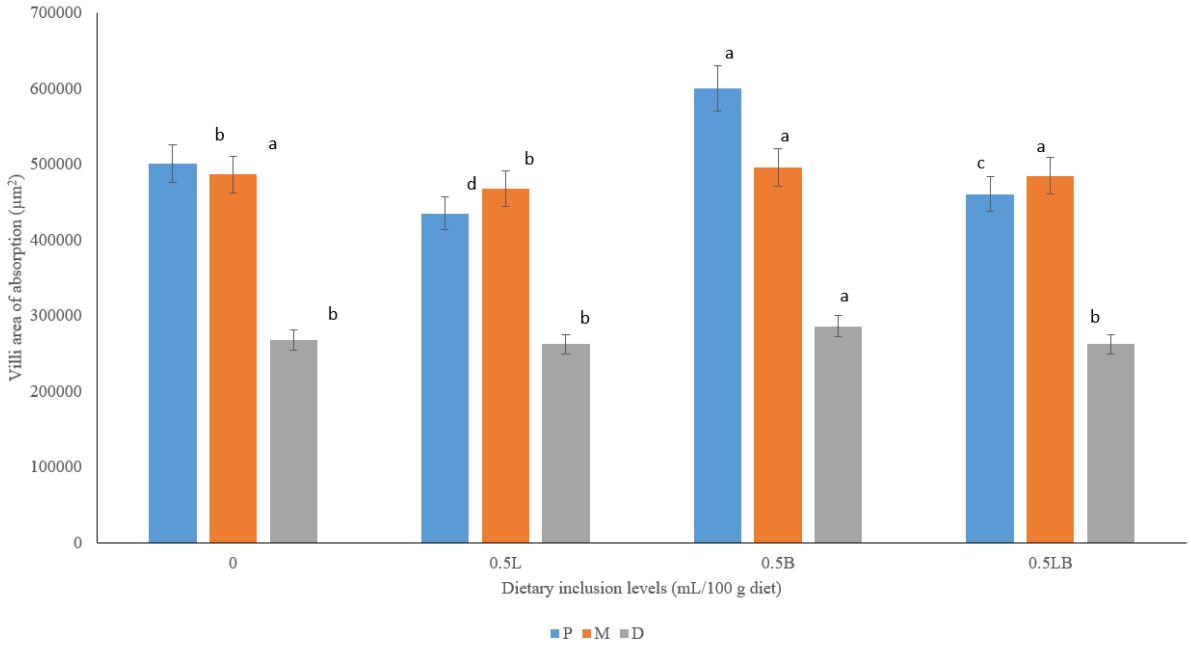

Figure 3: Villi absorption area of *Clarias gariepinus* fed diets supplemented with African ebony leaf and stem-bark extracts for 8 weeks

Similar bars with different letters are significantly different at $P < 0.05$
L, Leaf; B, Bark; LB, Leaf + Stem-Bark; P, Proximal region; M, Mid-region; D, Distal region

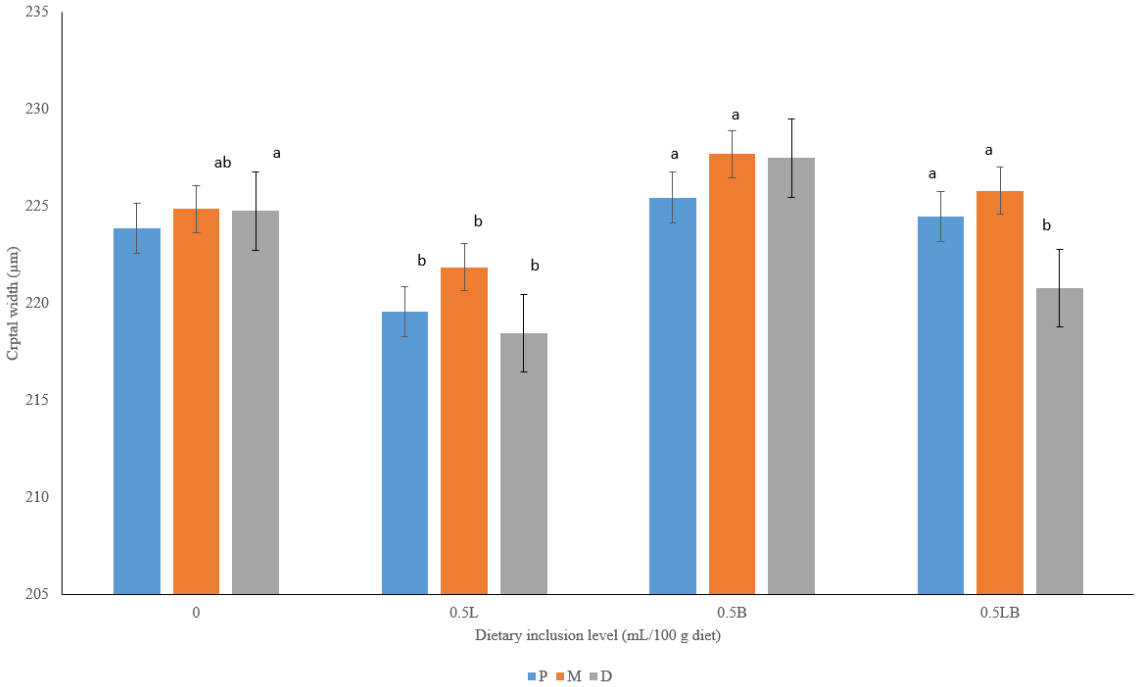

Figure 4: Villi cryptal width of *Clarias gariepinus* fed diets supplemented with African ebony leaf and stem-bark extracts for 8 weeks
Similar bars with different letters are significantly different at $P < 0.05$
L, Leaf; B, Bark; LB, Leaf + Stem-Bark; P, Proximal region; M, Mid-region; D, Distal region

DISCUSSION

Phytobiotics have been considered to be a significant bio-resource in aquaculture due to their growth-promoting and immune-stimulating effects in aquaculture (Dadras *et al*., 2023). The phytochemical screening confirmed the presence of key bioactive metabolites, including flavonoids, alkaloids, saponins and tannins, in both AE leaf and stem-bark extracts. However, the leaf extracts exhibited consistently higher quantities of tannins, saponins and alkaloids, whereas the stem-bark extracts, particularly the ethanol extract, showed elevated flavonoid concentrations. Flavonoids are well-recognized for their antioxidant, antimicrobial and gut-protective functions, which have been linked to improved nutrient absorption and intestinal integrity in fish (Radwan *et al.,* 2022). These compositional differences likely underlie the superior physiological performance observed in fish fed stem-bark extract diets.

The present study evaluated the effects of dietary supplementation of *Diospyros mespiliformis* (African ebony; AE) leaf and stem-bark extracts on growth performance, nutrient utilization, and intestinal histomorphology of *Clarias gariepinus*. The findings demonstrated that AE stem-bark extract at 0.5 mL/100 g diet produced superior outcomes across most performance and gut morphological indices compared to the leaf and combined extracts. The observed differences highlight the varied phytochemical profiles of leaf and bark materials and their distinct physiological impacts on fish. The wider villi area of absorption recorded in the fish fed AE stem-bark extract could have contributed to higher absorption of nutrients with resultant improved weight gain, specific growth rate, better feed conversion ratio and protein efficiency ratio relative to other treatments. This aligns with earlier studies where phytogenic additives enhanced digestive efficiency, enzyme secretion and intestinal morphology, thereby supporting better nutrient assimilation and growth (Adeniyi *et al.,* 2022, 2025; Adegbesan *et al.,* 2020; Kolygas *et al.,* 2025). The higher flavonoid content in the stem-bark extract may have reduced oxidative stress and supported intestinal function, resulting in improved feed utilization. The observations on the enhanced growth performance of the AE stem-bark coincides with earlier researchers (Adeshina *et al*., 2021; Yue *et al*., 2024; Adeniyi *et al*., 2023, 2025). Conversely, fish fed the AE leaf extract showed the lowest performance indices, which could be associated to the higher concentrations of tannins and saponins. Tannins and saponins are known to form indigestible complexes with proteins and minerals, and impair nutrient digestibility when present in elevated quantities (Francis, 2001; Omnes *et al*., 2017; Li *et al*., 2020) and could be responsible for lower feed intake obtained in the fish fed AE leaf extract in the current study. Thus, while both plant parts contain bioactive compounds, the relative balance of enhancing (flavonoids) and antinutritional (tannins) factors may determine growth outcomes.

The midgut (anterior intestine) of fish is usually divided based on their location into three: the proximal, middle, and distal regions (Olsson, 2024). Intestinal villus height and width are key indicators of gut absorptive surface area and digestive efficiency. The significantly higher villus height and width observed in fish fed AE stem-bark extract, particularly in the proximal and mid-intestinal

region, suggest enhanced nutrient absorption capacity. Enhanced villus architecture is commonly associated with the action of phytochemicals that improve mucosal health and modulate gut microflora (Radwan *et al.,* 2022) which promote digestion and consequently growth performance. The reduced villus dimensions in fish fed AE leaf extract diets correspond with their lower growth performance. This again supports the influence of higher tannin levels, which may impair mucosal development, reduce the contact areas between mucosal epithelial cells and the digested food (Zhang *et al*., 2020), hence, reduce nutrient availability for enterocyte renewal. Similarly, villus absorption area and cryptal width were superior in fish fed the stem-bark extract, reinforcing the improved intestinal functionality. The cryptal width patterns indicate active cellular proliferation supporting villus maintenance, which is essential for optimal nutrient uptake. The present findings provide experimental evidence supporting the use of *D. mespiliformis* stem-bark extract as a natural growth promoter and gut health enhancer in *C. gariepinus*. The observed improvements in intestinal morphology are consistent with reports that phytobiotics modulate intestinal integrity and digestive physiology (Adegbesan *et al.,* 2020; Adeniyi *et al*., 2023; Sandeep *et al.,* 2025).

CONCLUSION

Overall, dietary supplementation with AE stem-bark extract at 0.5 mL/100 g diet significantly improved growth performance, feed utilization and intestinal morphology in African catfish. Given growing concerns about antibiotic resistance and chemical residues in aquaculture, AE stem-bark extract represents a safe, locally available phytogenic alternative with clear benefits. The findings in this study showed that 0.5 mL ethanol extract of *D. mespiliformis* stem-bark /100 g basal diet is capable of enhancing growth performance and hence it is recommended as herbal supplement in the production of the African catfish. Further studies should investigate optimal inclusion levels, bioavailability, and immunomodulatory effects to support broader application in commercial aquafeeds.

AUTHORS CONTRIBUTIONS

IMM and OVA designed the study; IMM conducted the experiment, collected the data and wrote the first draft of the manuscript; OVA supervised the experiment, analyzed the data, and revised the manuscript. All authors approved the manuscript for submission.

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